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BIOLOGY AND IMMATURE STAGES OF TWO SPECIES OF *HYDROPTILA* DALMAN (TRICHOPTERA: HYDROPTILIDAE) WHICH CONSUME *CLADOPHORA* (CHLOROPHYTA)

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Abstract.—Adults of *Hydroptila armata* Ross and *H. perdita* Morton (Trichoptera: Hydroptilidae) are often taken near streams, but information on their immature stages is lacking. Field-collected females laid eggs in the laboratory, and the larvae were reared to the adult stage. Larvae of both species consumed the liquid contents of individual cells of *Cladophora* (Chlorophyta) by piercing cell walls with their asymmetrical mandibles; early instars of *H. armata* also occasionally removed epiphytic diatoms and consumed them. Final instars of both species used algal filaments and silk secretions to construct cases, and *H. perdita* incorporated mineral and detrital material as well. Hypermetamorphosis was exhibited by each species; instars 1–4 were completed rapidly (2–6 d each), whereas the final stadium was of longer duration (9–16 d). Cases were attached to rocks and sealed prior to pupation. The total duration of the immature stages (egg, larva, pupa) under laboratory conditions (~20°C; 12:12 L/D) was 43–54 d for *H. armata* and 36–47 d for *H. perdita*. The immature stages are described.

Key Words: *Hydroptila armata, Hydroptila perdita,* microcaddisflies, aquatic insects, streams, algae, *Cladophora,* rearing

The immature stages of most species of Trichoptera are unknown despite ample attention by aquatic entomologists (Wiggins 1990, 1996). The species richness of microcaddisflies (Hydroptilidae) is second only to the Limnephilidae, with some 311 species reported from North America, Mexico, and Greenland (Morse 1993). A number of studies of larval biology and morphology of Nearctic species have been conducted (Ross 1944, Flint 1962, Flint and Herrman 1976, McAuliffe 1982, Vaillant 1984, Huryn 1985, Resh and Houp 1986, English and Hamilton 1986, Keiper et al. 1998, Keiper and Foote 1998), but descriptions of the immature stages which include the early instars have not been published. The Australian (Wells 1985), European (Nielsen 1948, Lepneva 1964, Solem 1972), and Japanese (Ito and Kawamura 1980) hydroptilid faunas are somewhat better understood.

Hydroptila Dalman contains over 100 species from North America (Morse 1993). Ross (1944) gave brief descriptions of the fifth instars of a number of species from Illinois, including *H. armata* Ross; the larva of *H. perdita* Morton was previously unknown. Adults of both species have been collected near streams (Ross 1944, Huryn and Foote 1983). Herein, we give biological and morphological information on *H. armata* and *H. perdita* obtained during laboratory rearings initiated from eggs laid by field-collected females.

Materials and Methods

To initiate rearing, adult females were taken with an aspirator from a sheet illuminated with an ultraviolet collecting light. Collecting vials (containing live females) were placed in a cooler with ice for transport back to the laboratory. *Hydroptila armata* was taken at the Hocking River, 1 km west of Nelsonville, Ohio (Athens Co.), and *H. perdita* was collected at the Little Miami River near Fort Ancient State Memorial (Ohio, Warren Co.).

In the laboratory, adults were narcotized with CO_2, and females were pierced through the mesosternum with a number 3 insect pin when signs of revival were noted. This caused some females to dump their eggs in a mass or chain, and we transferred these adults with their eggs to individual Petri dishes containing stream water. Females were allowed to float on the surface overnight until the completion of egg laying, and then preserved in 70% ethanol. Eggs were also allowed to float on the surface until larval eye spots appeared. Small masses of the filamentous alga *Cladophora* (Chlorophyta) collected from local streams were rinsed gently with distilled water to remove any invertebrates present, placed in the dishes, and the eggs submerged among the filaments. All Petri dishes were kept at ~20° C, and a 12:12 light : dark photoperiod maintained.

Larval behavior and feeding habits were observed at 6–50× with a Wild M5 dissecting microscope. Early instars (1–4) were determined by direct observation of molts. If enough larvae were available, representatives of each stadium were collected, fixed with Kahle's solution, and preserved in 70% ethanol following the methods of Wiggins (1996). The water was changed every other day, and the algal food source was replenished when necessary. Upon attaining the fifth (and final) instar, larvae were transferred to aerated rearing chambers (Keiper and Foote 1996) with stream water, small rocks, algae, and mineral and detrital material to facilitate case building.

Illustrations of the immature stages were initiated by obtaining a tagged image format (TIF) computer file using a low light camera (Optronics Engineering DEI-470) mounted on the Wild scope or a Leica compound microscope, and Image Pro Plus image analysis software for IBM. To acquire images at high power (100×), larvae were placed on a microscope slide to which a drop of glycerol was added and viewed with the compound scope. TIF images were printed and traced or used as a reference. Measurements were obtained using the Image Pro Plus software. Physical descriptions and head capsule width measurements were taken from 10 specimens for each instar, and other measurements were obtained from one specimen per instar.

Results

Biology of *Hydroptila armata* Ross

Approximately 100 eggs were obtained from the single *H. armata* induced to oviposit. Eggs were ovoid, colorless, without surface markings, and measured 0.150 × 0.135 mm (n = 15). Eggs were laid in chains, but spread singly over the surface of the water when the female was placed in the Petri dish. After 5 d of incubation, the developing embryo was observed clearly through the chorion, and dark eye spots became apparent after 6 d. After 7 d, dark setae were seen pressed against the inside of the chorion, and the first hatching occurred approximately 12–24 h after these setae were first observed. The remaining eggs hatched after 8 d, giving an incubation period of 7–8 d at ~20°C.

First instars moved among filaments of *Cladophora*, and appeared to feed almost exclusively on the apical cells of filaments. Larvae consumed the contents of algal cells by piercing them with their mandibles and removing the fluid protoplast. Early instars executed up to 25 bites before piercing a cell and obtaining its contents. One larva

grasped a cell with its mandibles in a symmetrical orientation, and quickly pulled its head away, tearing off a piece of the cell wall. This was discarded, and the larva resumed its series of bites.

Several larvae used their mandibles to occasionally remove epiphytic diatoms from filaments of *Cladophora* by placing the tips of their mandibles on both ends of the elliptical frustule, and prying it from the filament. Larval guts were consistently dark green from consumption of *Cladophora* protoplast, therefore it appears that diatoms constituted only a minor proportion of the diet of *H. armata.*

Fifth instars constructed cases composed of two valves, similar in shape to that illustrated by Wiggins (1996). Approximately 85% of the case material was short filaments of *Cladophora,* and only 15% was mineral particles bound together by silken secretions. Larvae occasionally "unstitched" the ventral edge of their cases, and added more material before reattaching the valves, thus accommodating the increasing girth of the abdomen as the larva accumulated food reserves (see Nielsen 1948).

Completed cases were approximately 3.4 mm in length, and ranged from 0.735–0.887 mm in height. Larvae laid down layers of silken secretions to form a cocoon on the inside walls of cases. These bouts of activity were executed an undetermined number of times prior to attaching and sealing their cases, each lasting approximately 3–10 minutes. Bouts were spread out over a period of several days.

Fifth instars pierced individual cells within filaments of *Cladophora* with their asymmetrical mandibles (see below); the right mandible is pointed and was used to puncture individual cells, while the left one has a serrated inner edge, and apparently was used to maintain a grip on the cell when a biting motion was executed. During a bite, each mandible adducted to different degrees. The point of the right mandible was inserted into the cell wall and adducted strongly. In contrast, the inner edge of the left mandible adducted slightly, pressing the cell to the mouth of the larva. A bite was facilitated with a counterclockwise twist of the head (when viewed dorsally) approximately 20–30°, which applied leverage to the force of the right mandible. Epiphytic diatom consumption by final instars was not observed.

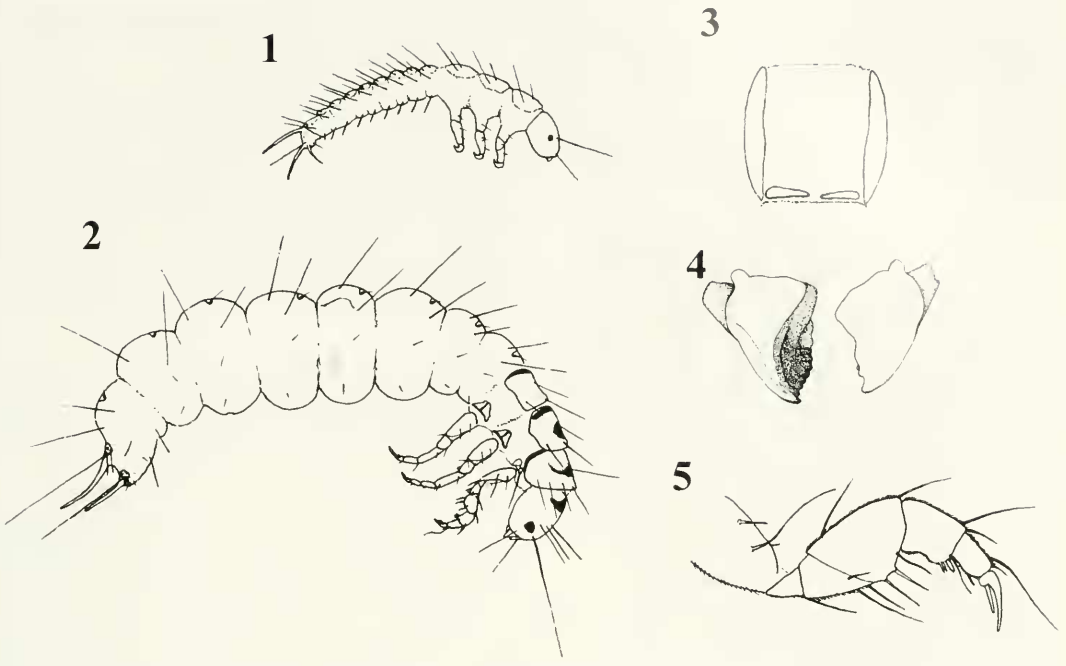
The duration of the five instars under laboratory conditions was 4–6, 5–6, 4–6, 4–6, and 12–16 d, while the pupal duration was 7–8 d. The total duration of the immature stages (egg, larva, and pupa) was 43–54 d, with a larval/pupal period of 36–48 d.

Biology of *Hydroptila perdita* Morton

Three females induced to oviposit produced 11, 115, and 175 ($\bar{x}$ = 100.3) eggs, respectively, which were scattered loosely over the water surface. The first eggs hatched after 9–12 d of incubation. The newly-hatched larvae immediately began to feed mostly on the contents of the apical cells within filaments of *Cladophora.* All instars fed in a manner similar to that described for *H. armata,* although epiphytic diatom consumption was never observed.

Case building commenced within 24 h of molting into the fifth instar. This species appeared to be generalized in its case material requirements, utilizing algal filaments, sand grains, and detrital material in approximately equal amounts. One individual even incorporated what seemed to be a blue carpet or clothing fiber which fell into the rearing chamber. Otherwise, construction and shape resembled that of *H. armata.* Larvae attached their cases to stones within the rearing chambers or the rearing chamber wall and floor, and sealed them prior to pupation.

The duration of the five instars under laboratory conditions was 2–4, 3–4, 2–3, 2–3, and 9–12 d, while the pupal duration was 9–10 d. The total duration of the immature stages (egg, larva, and pupa) was 36–47 d, with a larval/pupal period of 27–36 d.



Figs. 1–5. *Hydroptila armata*. 1, Third instar, lateral view. 2, Fifth instar, lateral view. 3, Prosternum, anterior up. 4, Left and right mandibles, ventral view. 5, Fore leg, lateral view.

Description of Larval *Hydroptila armata* Ross

First two instars with colorless, unmarked head capsules, black eye spots, and black setation; bodies dorsoventrally flattened. Instars three and four with slight but noticeable darkening of sclerites. Body round in cross section. Head capsule and thoracic nota covered with dense pile visible at high magnification only. Head capsule dimensions given in Table 1.

First instar.—Total length, 0.692 mm; prothorax, 0.070 mm; mesothorax, 0.073 mm; metathorax 0.086 mm; abdominal segment 1, 0.034 mm; segment 2, 0.034 mm; segment 3, 0.033 mm; segment 4, 0.035 mm; segment 5, 0.030 mm; segment 6, 0.032 mm; segment 7, 0.038 mm; segment 8, 0.040 mm; segments 9 and 10, 0.042 mm.

Second instar.—Measurements not taken, but size increase proportional to first instar.

Third instar.—Total length, 2.260 mm; prothorax, 0.224 mm; mesothorax, 0.258

Table 1. Head width range, median, and factor of increase[1] of *H. armata* and *H. perdita*.

Instar	*H. armata*			*H. perdita*		
	Head Width (mm)		Factor of Increase	Head Width (mm)		Factor of Increase
	Range	Median		Range	Median	
I	0.090	0.090		0.090	0.090	
II	0.105–0.120	0.113	1.26	0.120	0.120	1.33
III	0.150	0.150	1.33	0.150	0.150	1.25
IV	0.180–0.195	0.188	1.25	0.180–0.195	0.188	1.25
V	0.210–0.225	0.218	1.16	0.210–0.240	0.225	1.20

[1] Obtained by dividing the head width median of the current instar by that of the previous instar.

mm; metathorax, 0.224 mm; abdominal segment 1, 0.125; segment 2, 0.125; segment 3, 0.143; segment 4, 0.110 mm; segment 5, 0.130 mm; segment 6, 0.121 mm; segment 7, 0.139 mm; segment 8, 0.130 mm; segments 9 and 10, 0.192 mm (Fig. 1).

Fourth instar.—(Only one specimen obtained, and placed in 70% ethanol which appears to have caused the specimen to contract.) Total length, 1.861 mm; prothorax, 0.258 mm; mesothorax, 0.177 mm; metathorax, 0.157 mm; abdominal segment 1, 0.078 mm; segment 2, 0.089 mm; segment 3, 0.063 mm; segment 4, 0.051 mm; segment 5, 0.087; segment 6, 0.066 mm; segment 7, 0.113 mm; segment 8, 0.128 mm; segments 9 and 10, 0.172 mm.

Fifth instar.—Total length, 4.022 mm; prothorax, 0.094 mm; mesothorax, 0.102 mm; metathorax, 0.137 mm; abdominal segment 1, 0.085 mm; segment 2, 0.102 mm; segment 3, 0.137 mm; segment 4, 0.599 mm; segment 5, 0.609 mm; segment 6, 0.665 mm; segment 7, 0.466 mm; segment 8, 0.381 mm; segments 9 and 10, 0.463 mm. Head capsule and thoracic nota with yellowish-brown base color and black banding pattern (Fig. 2); recently-molted fifth instars without dark banding pattern on thoracic sclerites and head capsule. Two small triangular prosternal sclerites posteriorly, concolorous with other sclerites (Fig. 3). Mandibles asymmetrical and without setae; right mandible pointed apically with small subapical tooth; left one with inner edge finely serrated (Fig. 4). Fore tarsus with a long subapical dorsal seta, approximately twice length of tarsal claw; basal seta of tarsal claw extending past midpoint of claw (Fig. 5). Meso- and metapleura with dark brown sutures. Abdomen of the mature larva approximately two to three times girth of thorax; sclerites of abdominal segments 9 and 10 pale; anal claw dark brown. Dorsal chloride epithelia elliptical and faint. Primary setae very dark basally, gradually becoming light brown apically.

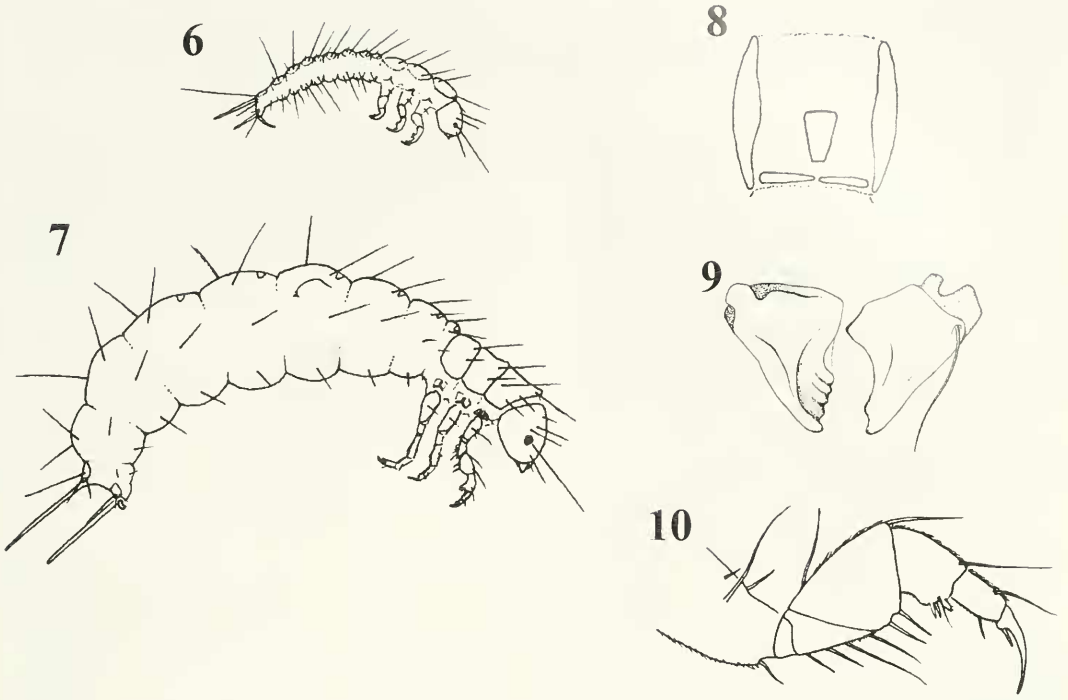
Description of Larval *Hydroptila perdita* Morton

Bodies of instars one and two somewhat flattened dorsoventrally. Instars three and four with slight but noticeable darkening of sclerites; body round in cross section. Head capsule and thoracic nota covered with dense pile visible at high magnification only. Head capsule dimensions given in Table 1.

First instar.—Total length, 0.570 mm; prothorax, 0.070 mm; mesothorax, 0.070 mm; metathorax, 0.050 mm; abdominal segment 1, 0.021 mm; segment 2, 0.024 mm; segment 3, 0.033 mm; segment 4, 0.039 mm; segment 5, 0.027 mm; segment 6, 0.023 mm; segment 7, 0.025 mm; segment 8, 0.036 mm; segments 9 and 10, 0.036 mm; central gill, 0.135 mm; lateral gills, 0.150 mm (Fig. 6). Head capsule uniformly dull yellow, unmarked, and darker than rest of body; eye spots dark; setae black.

Second, third, and fourth instars.—Measurements not taken, but size increase proportional in successive instars. Head capsule yellowish, darkening slightly with age.

Fifth instar.—Total length, 2.62 mm; prothorax, 0.150 mm; mesothorax, 0.125 mm; metathorax, 0.140 mm; abdominal segment 1, 0.070 mm; segment 2, 0.170 mm; segment 3, 0.215 mm; segment 4, 0.305 mm; segment 5, 0.285 mm; segment 6, 0.265 mm; segment 7, 0.215 mm; segment 8, 0.250 mm; segments 9 and 10, 0.165 mm; central gill, 0.458 mm; lateral gills, 0.410 mm. Head capsule uniformly dull yellow and unmarked (Fig. 7). Three prosternal sclerites (Fig. 8); posterior sclerites triangular and narrow; central sclerite quadrangular and tapering posteriorly; concolorous with other sclerites. Mandibles asymmetrical; right pointed apically, with small subapical tooth, and one seta at posterolateral corner; left with coarsely serrated inner edge, and without setae (Fig. 9). Fore tarsus with short subapical dorsal seta, approximately length of tarsal claw; basal seta of



Figs. 6–10. *Hydroptila perdita*. 6, First instar, lateral view. 7, Fifth instar, lateral view. 8, Prosternum, anterior up. 9, Left and right mandibles, ventral view. 10, Fore leg, lateral view.

tarsal claw extending up to midpoint of claw (Fig. 10). Meso- and metapleura with dark brown sutures. Abdomen of mature larva approximately twice the girth of thorax. Sclerites of abdominal segments 9 and 10 and anal claw brown. Dorsal chloride epithelia elliptical and faint. Primary setae very dark basally, gradually becoming light brown apically.

Pupa.—Appears similar to those species described by Nielsen (1948) and Ito and Kawamura (1980). Total length, 2.32 mm; head width, 0.63 mm; antennal length, 1.28 mm.

Discussion

Only a few descriptions of larval *Hydroptila* are available. Ross (1944) separated groups of species from Illinois based on color of head and thoracic sclerites, but cautioned that color patterns are variable within species. His key is presently unreliable due to species whose larvae are unknown, new distributional records, and recent reports of species new to science from eastern North America (e.g., Houp et al. 1998). *Hydroptila armata* is easily distinguished from *H. perdita* based on the dark color pattern of the head and thorax, lack of a central prosternal sclerite, presence of a long subapical tarsal seta, and lack of setae on the posterolateral corner of the right mandible of *H. armata*. No characters were found to separate the early instars of these species, however the early instars of *H. itoi* Kobayashi have brown thoracic and abdominal sclerites with long setae (Ito and Kawamura 1980) which contrast those observed on *H. armata* and *H. perdita*.

Few morphological characters appear to be of general use for separating final instar *Hydroptila*. Prosternal sclerites appear variable in number (2 or 3) between species; if present, the shape of the central sclerite varies from trapezoidal (*H. perdita*), diamond-shaped (*H. tineoides* Dalman) (Niel-

sen 1948 as *H. femoralis* Eaton), and pentagonal (*H. coweetensis* Huryn) (Huryn 1985). Mandible morphology of *Hydroptila* may be the most useful character for species identification of larvae because shape and setation appear to differ among species (Nielsen 1948, Lepneva 1964, Ito and Kawamura 1980, Huryn 1985, Keiper 1998, Keiper and Foote 1998, this study). Further larva-adult associations through rearings coupled with detailed distributional records should facilitate the compilation of regional keys to species based primarily on mandible morphology for *Hydroptila* and possibly other microcaddisfly genera.

Previous investigations indicated that some degree of trophic specialization occurs in hydroptilid larvae (Nielsen 1948, Resh and Houp 1986). Recently, Keiper et al. (1998) demonstrated that *H. waubesiana* Betten and *Oxyethira pallida* (Banks) completed the larval/pupal period when given only monocultures of certain algal taxa, whereas larvae consuming other algal monocultures did not. Although we gave larvae only *Cladophora* during this investigation, we suspect that *H. armata* and *H. perdita* exhibit a similar degree of feeding specialization. The mandibles of each species appear similar to those of *H. waubesiana* (Keiper 1998), and are apparently specialized for piercing individual cells of green algae.

The collection localities for adults utilized during this study corroborate statements by Ross (1944) that each of these species prefers moderate-sized streams. Ross (1944) collected *H. armata* fifth instars in Illinois streams, but the larval microhabitat of *H. perdita* remains unknown. Our observations of larval behavior and feeding habits suggest that larvae of *H. perdita* inhabit rock substrates with ample growths of *Cladophora,* as this is where similar species have been taken (e.g., Huryn 1985, Wells 1985, Keiper 1998, Keiper and Foote 1998). Further collections of fifth instar *Hydroptila* for laboratory rearings in areas where *H. perdita* adults are encountered are needed to confirm this.

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